

tures being at least partially defined by a gasket between the electrode support and the second cartridge component); see the discussion of FIG. 1c.

[0133] Since it was believed that using the electrode-pairing scheme might result in the assay on a previously used working electrode affecting its function as the counter electrode for the next working electrode, an experiment was devised wherein three different protein coatings were used to determine their effect. The effects of three protein coatings were measured: avidin, CK-MB capture antibody, and Bovine IgG. The ECL of a 10 nM ruthenium-tris-bipyridine solution in a tripropylamine-containing buffer was measured on non-coated electrodes with various counter electrodes (coated, non-coated, fired, and virgin); these results are listed in Table 2. In this table ECL_{fired CE} denotes the ECL from the working electrode when paired with a counter electrode that has been previously fired as a working electrode and ECL_{virgin CE} is for ECL from the working electrode when paired with a counter electrode that has not been previously fired as a working electrode. The observed ECL signals were all within experimental error of one another demonstrating the unexpected result that neither the presence of protein on the surface nor the prior use as a working electrode had any effect on the performance of that surface as a counter electrode.

TABLE 2

Effects of Protein Coating and Application of Oxidative Potentials to Electrodes Previously Used as a Counter Electrode in Free TAG ECL Generation		
Protein on C.E.	ECL _{fired CE}	ECL _{virgin CE}
anti-CK-MB	199	207
Blank	199	197
Avidin	181	205
IgG	203	214

[0134] With reference to FIG. 4, and by way of example only, operation of a simplified electrode array employing the pair-wise firing scheme within a single detection chamber will be described. For purposes of this operational example, introduction of sample, assay reagent(s), wash solution(s) and/or buffer(s) through the fluid input line 450 will not be discussed; it is to be understood that each of the necessary constituents for performing the assay are present in the detection chamber for this example. At least one of the electrodes will operate as a dedicated counter electrode, e.g., 401, and will therefore not have any assay reagents immobilized thereon. Electrodes 402-407 will have assay reagents immobilized thereon; electrodes 402-406 are to be used as dual-role electrodes and electrode 407 is to be used as a dedicated working electrode. As pictured in the figure, the electrodes are preferably arranged in one dimensional arrays (most preferably, linear arrays) along the fluid path in the detection chamber. The dedicated counter electrode 401 will be used first in conjunction with the adjacent dual-role electrode 402, wherein the dual-role electrode will be operated as a working electrode to perform the desired assay at dual-role electrode 402. Thereafter, dual-role electrode 402 will be operated as a counter electrode and will be pair-wise fired with dual-role electrode 403, wherein dual-role electrode 403 will be operated as a working electrode to perform the desired assay at dual-role electrode 403. This pair-wise firing is continued for the remaining electrodes until electrode pair 406 and 407. This last remaining pair will operate dual-role electrode 406

as a counter electrode and dedicated working electrode 407 as a working electrode to perform the desired assay at dedicated working electrode 407. Preferably, the electrode pairs used in a specific firing are adjacent each other (i.e., there are no other electrodes located between them) to avoid the undesired emission of ECL from an electrode located in the intervening space.

[0135] The use of patterned electrodes in cartridges may impose certain unique design and/or performance constraints. In particular, the use of patterned electrode leads may lead to problems associated with voltage drops along the leads, especially in applications like electrochemiluminescence that often require relatively high currents. The problems are often greatest when using electrodes comprising thin layers of only moderately conductive materials such as carbon inks. The problem may be partially mitigated by use of multi-layer patterned electrodes (where the conductivity of an exposed moderately conductive material such as a carbon ink is increased by printing it over a more conductive material such as a silver ink) although this approach introduces additional manufacturing steps. Alternatively, the problem may be partially mitigated in systems having multiple assay electrodes by keeping the leads short (preferably, so that the resistance between the electrode and the electrical contact is less than 500 ohms, more preferably less than 300 ohms, most preferably less than 100 ohms) to minimize the voltage drop and by keeping the leads about the same length to make the voltage drop consistent from electrode to electrode.

[0136] In an assay module comprising multiple working electrodes, the variability from electrode to electrode in the voltage drop across the electrode leads is preferably smaller than the potential applied during the course of an assay measurement so that this variability has minimal effect on the variability of the measurements. In especially preferred embodiments, the variability in voltage drop across the leads is less than 20% of the potential applied during the course of an assay measurement, more preferably less than 10% or most preferably less than 2%. Alternatively, the uniformity in leads can be described in terms of the variation in resistance across the leads which is preferably less than 50 ohms, more preferably less than 10 ohms, most preferably less than 1 ohm.

[0137] Where the arrangement of the electrodes and/or contacts makes it difficult to keep the leads a uniform length, the matching of lead resistances can be accomplished by geometrically matching the length-to-width ratio of each electrode lead (assuming consistent print thickness). This length-to-width ratio is referred to hereinafter as the "number of squares". Typically, for a preferred cartridge-based configuration using screen printed carbon inks, the electrode leads are on the order of 4 to 5 squares. Commercially available inks typically have ink resistances that are specified in resistance per square per thickness (e.g., ohms/square/mil) and can vary widely depending on the ink selected. In a particularly preferred embodiment, a carbon ink is used that possesses an ink resistance that measures approximately 15 ohms/square/mil. The total resistance measured from end-to-end across a lead for one preferred embodiment is typically on the order of 450 ohms for a configuration utilizing a 5 squares lead.

[0138] FIG. 2 depicts one preferred embodiment of an addressable electrode array for generating ECL that can be incorporated into a cartridge-based form factor having the requisite provisioning for sample/reagent mixing/delivery.